SYMPOSIUM ON PROBLEMS IN THE MICROBIOLOGICAL ANALYSIS OF FOODS[1]

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Microbiological analyses of foods have been conducted for several decades but the fundamental problems of philosophy and methods have not been solved to the satisfaction of research, production and legislative or control agencies.

Dr. Dack discussed the philosophy of the microbiological analysis of foods, which in part demands that enforcement agencies educate processor groups for the greatest over-all gain in food processing. It is also essential to recognize the sources from which contaminating organisms arise and to understand the selective effect of temperature, moisture, pH and processing procedures. Bacteria may be contributed by food processing lines, in which case sanitation and decontamination must be maximum to reduce counts. Pathogens, viruses, as well as enteric bacteria, may be contributed via fecally polluted hands of a food handler and *Micrococcus pyogenes* var. *aureus* via skin lesions. Certain pathogens, *e.g. Salmonella*, are naturally present in some foods, such as frozen, diced eggs and meat. The low incidence of enteric disease is due to cooking of foods prior to eating, and to the fact that animals with septicemia do not enter the meat supply in federally inspected processing plants. Established coliform tests for water are not applicable to foods, except where these organisms are found in foods previously heated to temperatures sufficient to destroy vegetative cells. Total bacterial count does not measure safety of foods but should be part of quality control procedures.

Dr. Gunderson discussing sampling problems in foods showed that there was no direct relationship between microbiological counts in a food product as finished at the processing plant and that purchased by the consumer. High counts may be produced by improper temperatures of storage. No increase in numbers of psychrophilic or mesophilic bacteria occurred in chicken pies stored at −25 C even after 3 months of storage. When similar products were stored at 0 C the psychrophilic and mesophilic counts increased, the former more slowly at first, but at 24 days the numbers were approximately equal having increased from 15 to 25 thousand to over 1 billion with the product spoiling. At 5 C psychrophilic count increased more slowly for the first 5 days after which there was a rapid increase in count. At 10 days counts had increased with spoilage to 3 to 6 billion with the greater numbers representing the mesophilic microorganisms. At 20 C similar products spoiled in 2 days with the mesophilic count being appreciably higher than the psychrophilic at all stages. Chicken pies inoculated with large numbers of microorganisms (1.5 billion *Escherichia coli*, 2.8 billion *Streptococcus faecalis*, 15 million *Micrococcus pyogenes* var. *aureus*, 33 million gram positive rods per g) showed little change after freezing for 24 hr but after baking all counts dropped to zero except the last which also approximated this figure. Microbiological counts of less than 100,000 bacteria per g of product are readily obtained in commercial processing of most products.

Dr. Silliker discussed the selection of bacteriological methods showing that bacteriological methods are less precise than analytical chemical methods for most foods and that the significance of the bacteriological methods is not as readily interpreted as the chemical. He stated that

[1] This symposium was presented at the Fifty-seventh General Meeting of the Society of American Bacteriologists at Detroit, Michigan, on April 30, 1957. Participants were: Gail M. Dack, *Food Research Institute, The University of Chicago, Chicago, Illinois;* M. F. Gunderson, *C. A. Swanson and Sons, Omaha, Nebraska;* John H. Silliker, *Bacteriology Research Division, Swift and Company, Chicago, Illinois;* Betty C. Hobbs, *Food Hygiene Laboratory, Central Public Health Laboratory, London, England;* W. L. Mallman, *Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan;* F. S. Thatcher, *Microbiology Section, Food and Drug Directorate, Department of National Health and Welfare, Canada;* D. A. A. Mossel, *Central Institute for Nutrition Research, T. N. O., Utrecht, The Netherlands;* and R. Buttiaux, *Department of Food Microbiology, Pasteur Institute, Lille, France.*

neither the simple enumeration nor the isolation of specific types is sufficient for control but rather it is necessary to isolate specific groups of bacteria and arrive at a reasonably accurate estimate of their numbers. Since the specific type may be only a small portion of the total flora and also since the organisms may be damaged physiologically by the factors involved in processing, it is difficult to arrive at a quantitative determination of numbers in the presence of large amounts of foodstuffs using present bacteriological methods and media. Methods of bacteriological analysis applicable for other disciplines of bacteriology cannot necessarily be applied to the study of food bacteriology. Media that are selective in water analysis for lactose fermenting organisms are no longer selective when foods such as ice cream, frozen desserts or citrus juices containing large amounts of sucrose or dextrose are added as these latter sugars may be present at 1.0 per cent concentration and large numbers of false positive tests may result. Samples of food tested for *Salmonella* may appear negative when relatively large quantities of food material are inoculated into selenite F, tetrathionate or other selective media, yet be positive if small inocula are used. A test has been devised based on centrifugation at 1100 × G of the sample emulsified or suspended in an appropriate volume of sterile water and the reinoculation of the sediment containing 99.9 per cent of the bacteria into the test medium. Removal of the soluble materials in the supernatant removed factors interfering with the specificity of the selective medium. Dr. Silliker stated that it would be dangerous to assign specifications arbitrarily insofar as food standards are concerned in the absence of reproducible methods.

Dr. Hobbs discussed the sources and multiplication of pathogens in foods as an integral part of the microbiological analysis of foods emphasizing that public health measures such as chlorination of water, pasteurization and refrigeration of milk and ice cream mix, together with the laboratory control of enforced and recommended bacteriological standards have reduced the incidence of water, milk and food-borne disease. A number of common foodstuffs still exist for which there are no recognized methods of control, and these may contain organisms such as the *Salmonella*, *Micrococcus* or *Clostridium* groups which can produce typical gastrointestinal syndromes if certain high numerical levels are reached, in addition to infections produced by smaller numbers of dysentery and typhoid organisms. The sources of the 3 genera previously mentioned are: (a) human carriers; (b) live animal carriers; and (c) animal products used as foodstuffs. Human carrier rates of *Salmonella* are not known but outbreaks from this source occur each year and as the number of outbreaks increase the number of carriers will increase since these organisms may be harbored from several weeks to one or more years. Coagulase positive staphylococci are present in the nasal tracts of 50 to 60 per cent, and on the hands of 15 to 20 per cent, of the general population. Of these, only 10 to 20 per cent are the bacteriophage and serological types associated with food poisoning. Heat resistant *C. perfringens* associated with food poisoning outbreaks is carried by only 2 to 5 per cent of the normal population. Live animal carriers are important in that approximately 2 per cent of domestic animals and scavenger birds harbor *Salmonella*. Strains of *C. perfringens* associated with food poisoning are present in 15 to 20 per cent of dogs and cats examined, a slightly higher percentage of hogs and a low percentage (1.7) of cattle. Staphylococci were associated predominantly with the flora of the normal and abnormal udder. Animal products such as milk, eggs and meat may harbor pathogens and the latter products are more likely to contain *Salmonella* associated in some degree with manufacturing processes. Large total counts were closely associated with increased numbers of suspected cases of food poisoning. It is essential to measure total anaerobic as well as aerobic flora as *C. perfringens* poisoning may take place in the presence of low aerobic and high anaerobic counts.

Dr. Mallman discussed indicator species, methods and meanings, including a comprehensive discussion of the organisms that have been used to indicate pollution in water supplies and how the problem differs to some extent in the microbiological analysis of foods. The media involved in the detection of the organisms were also discussed, and the methods of evaluation of tests were indicated. There is a trend at the present time to use drop plate methods in preference to certain other techniques when working with indicator organisms. The longevity of the indicator organism varies greatly according to

surroundings, as to whether they are water or foods and in the latter instance whether environment is neutral or acid. When enterococci are tested the results depend in a great part upon the method of recovery and a comparison was made of certain of these methods. It is important to adapt the method to the product under analysis.

Dr. Thatcher in discussing the topic "What May Microbiological Standards Mean" showed the complexity and the subjectiveness involved. He indicated that one should never have regulations merely for regulations' sake. Standards must be workable and solely to produce quality products and reduce food-poisoning and food-borne infections. Microbiological counts of products alone cannot be depended upon to give desired control as staphylococcus food poisoning has occurred in products containing no viable staphylococci, salmonellosis has developed in infants utilizing coliform-free egg products containing less than one *Salmonella* per g, and technical difficulties preclude routine determination of all pathogenic bacteria (*Brucella* sp., etc.) and of possible viruses present. Even if foods contain certain pathogens or indicator species they do not necessarily cause disease since foods with *Salmonella*, *C. perfringens*, *E. coli*, paracolons, fecal streptococci and staphylococci may be found on the market with no associated outbreaks; however, foods of this type or consistently dirty foods are more likely to cause disease than consistently clean foods. Foods may be examined at the market or under processing conditions or both. Precooked frozen dinners, meat pies and factory poultry present special problems, particularly as to sampling, and consequently such foods should probably be controlled at the factory and, where essential, examined using standards established by industry inspired codes. Certain smaller industries have no bacteriologists or sanitarians for routine control and may be ignorant and careless in public health and hygiene. Consequently, it might be better to control the products by administrative standards rather than by legally promulgated standards so a certain elasticity could be attained. Additional studies of specified groups of organisms, ecological competition, the effects of processing and the effect of storage are necessary before widespread restrictive legislation is applied. A major role of microbiological standards should be to indicate levels of contamination at which pressures need to be applied in order to improve sanitation and hygiene at the factory. Considerations of practicality and economics require that microbiological standards should be used only when experience indicates a need in terms of public health or spoilage.

Dr. Mossel discussed for Dr. Buttiaux and the other members of the International Association of Microbiological Societies (I.A.M.S) Committee the European approach to protecting the microbiological safety of foods, stressing the importance of recognizing that foods substandard from bacteriological aspects may be more important as a risk to consumer health than food substandard from chemical composition. The foods for which epidemological data point to necessity of more strict control are raw foods (raw sausage, frozen eggs, etc.); pasteurized foods (smoked sausage, dehydrated milk, ice cream, frozen meats) and canned foods. To protect the microbiological safety of foods strict codes for the preparation must be promulgated and inspection should be instituted to be certain that the codes are carried out properly. Inspection should be particularly vigilant against the use of contaminated raw materials, improper handling, inadequate processing and lack of refrigeration. Inspection should be strongly supported by education and punitive measures taken only where education fails. Regular samples should be taken for the laboratory but it must be realized that safe samples do not necessarily mean a safe product. Standard methods must be available before standards can be promulgated and the Section for Food Microbiology and Hygiene of the I.A.M.S. might be helpful in standardizing at the international level. The general approach to drafting microbiological standards for foods should be quantitative, specific and realistic. If the standards cannot be attained by industry they will be ignored or evaded. Drs. Buttiaux and Mossel recommend that standards should be different for: (a) fresh foods not obtained by microbial fermentation; (b) fresh foods produced by microbial fermentation; (c) semipreserved, canned, low-acid foods; and (d) "commercially sterile" canned foods. Standards proposed for the first group include freedom from pathogens, toxinogenic bacteria and certain indicator organisms, and illegally added antimicrobial substances, while aerobic and anaerobic plate counts shall not be over 10^5/g. Standards proposed for group

b include the same except the plate count is of proteolytic aerobic and anaerobic bacteria and not to be over 10^4/g. Standards proposed for group c are that there shall be no appreciable change in taste, odor, color, pH or consistency after incubation at 30 to 32 C for 5 days when the product is compared to unincubated cans. Other standards shall be as of group a. Proposed standards for "commercially sterile" canned foods include similar examinations after incubation at 30 to 32 C for 3 weeks and 50 C for 10 days. A detailed discussion of methods already used in Western Europe for the microbiological analysis of foods was given. These included aerobic plate count in tryptone dextrose yeast extract agar; proteolytic aerobic counts by drop plates on tryptone yeast extract gelatin agar; anaerobic counts by 2 methods; coliform counts in crystal violet-neutral red bile-lactose mannitol agar or McConkey's bile lactose peptone water with subsequent confirmation; fecal streptococci in Packer's crystal violet sodium azide blood agar at 39.5 C with subsequent confirmation; *Salmonella* by enrichment in both selenite and tetrathionate broths followed by confirmation; *M. aureus* on 7.5 per cent NaCl agar followed by confirmation and detection of illegally added preservatives and antibiotics by the *Saccharomyces cerevisiae* fermentation test.

Errors of sampling and failures to resuscitate organisms injured by processing must be recognized. Interpretation of data can only be made after careful study of all factors concerned with survival of organisms in foodstuffs.